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Research article

Impact of reference diet composition on apparent digestibility coefficients of two protein-rich ingredients in Nile tilapia

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ABSTRACT: Protein quality is related to amino acid composition and digestibility. Accurate evaluation of apparent digestibility coefficients (ADCs) of nutrients in commonly used feedstuffs is paramount for formulating efficient aquafeed. ADCs of soybean meal (SBM) and poultry by-product meal (PBM) were evaluated using reference diets formulated with two types of ingredients (semi-purified [SP] and practical [P]) for juvenile Nile tilapia (*Oreochromis niloticus*, Linnaeus) of the GIFT strain. Groups of 20 juveniles (65.05 ± 12.37 g) were fed twice a day to apparent satiety with one of the four experimental diets (SBM-SP, SBM-P, PBM-SP, and PBM-P) in quadruplicate for 30 days. After the last feeding, feces were collected by siphoning hourly and the ADCs of dry matter, protein, and amino acids (AAs) were calculated. Nile tilapia exhibited a high capacity to digest SBM and PBM, with most ADCs exceeding 90 %. The type of reference diet affected the ADCs of protein and AAs on the test ingredients, with the SP reference diet providing the highest ADC, mainly in SBM. Digestibility data generated with a P-type reference diet demonstrated more practical relevance than those generated with an SP-type reference diet. They can be applied in digestibility studies for Nile tilapia.

Keywords: *Oreochromis niloticus*, methodology, soybean meal, poultry by-product meal

Introduction

Accurate elucidation of apparent digestibility coefficients (ADCs) of commonly used protein-rich nutrient sources is vital to formulate nutrient- and cost-effective diets for commercial species (Fracalossi and Cyrino, 2013; Hardy, 2010; Lupatsch et al., 1997). In the evaluation of feed ingredient digestibility, the test ingredient is paired with a reference diet, usually at a ratio 30:70 (NRC, 2011). High-quality semi-purified (SP) ingredients with well-defined composition and without anti-nutritional factors are still used in some digestibility trials to compose the reference diet (Glencross et al., 2007; Lovell, 1998). Such ingredients predominantly provide one macronutrient and only trace amounts of vitamins and minerals; however, they can reduce palatability and feed intake (Hardy and Barrows, 2002) and do not represent the reality of commercial diets. In contrast, despite anti-nutritional factors, a reference diet composed of practical (P) ingredients has advantages, such as similar composition to commercial feeds, high feed consumption due to high palatability, and production of more fecal material (NRC, 2011).

Soybean meal (SBM) and poultry by-product meal (PBM) were the ingredients investigated in the present study because they are protein-rich sources widely used in commercial diets for Nile tilapia (*Oreochromis niloticus,* Linnaeus). SBM is abundantly available and a cost-effective source of digestible plant protein and essential amino acids (EAAs) (Gatlin et al., 2007; Nguyen et al., 2009). PBM is an animal source of highly digestible protein and a good alternative to fish meals because of its similar protein composition, high production volume, and relatively low cost (Cruz-Suárez et al., 2007; Hardy, 2010).

Nile tilapia is the second most-produced fish group in aquaculture worldwide, with a production of 4.4 million tons, and Brazil is the 4th largest producer (FAO, 2022a, b). Data on nutrient digestibility of Nile tilapia are available; nevertheless, the methodology varies and lacks a standard regarding the reference diet composition (Borghesi et al., 2008; Cardoso et al., 2021; Davies et al., 2011; Maas et al., 2019). Therefore, in the present study, we compared the use of different reference diet compositions (P or SP) in two important protein-rich feedstuffs to verify possible variations in digestibility of protein and AAs by Nile tilapia.

Materials and Methods

Feed ingredients and diet preparation

Two reference diets were formulated (Table 1) with semi-purified (SP) or practical (P) ingredients to meet the nutritional requirements of Nile tilapia (Pezzato et al., 2010) or *Oreochromis* sp. (NRC, 2011). Each reference diet was used to estimate nutrient digestibility of two practical protein-rich ingredients (Table 2), PBM and SBM, at a 30 % level of inclusion. The proximate composition, AAs, and energy content of the feed ingredients (corn, fish meal, and soy protein concentrate) were determined before the experimental diet formulation. The two reference diets (SP and P) and the four test diets (SBM-SP, SBM-P, PBM-SP, and PBM-P) contained 0.1 % of yttrium oxide as an inert marker.

Ingredients were ground using a hammer mill (1.0 mm screen mesh) and then manually sieved (0.6 mm), weighed, and homogenized in a horizontal mixer. The

Table 1 – Formulation of reference diets.

Ingredients (g kg^{-1} dry matter)	Reference diets	
	Semi-purified	Practical
Corn starch[1]	485.00	-
Casein[1]	283.00	-
Gelatin[1]	88.00	-
Cellulose[1]	43.00	-
Corn[2]	-	450.00
Fish meal[2]	-	350.00
Soy protein concentrate[3]	-	160.00
Soybean oil[4]	49.00	18.00
Dicalcium phosphate	20.00	-
Macromineral premix[5]	20.00	-
Micromineral and vitamin premix[6]	10.00	20.00
Choline[6]	1.00	1.00
Yttrium oxide[7]	1.00	1.00

[1]Produced by Rhoster; [2]Corn (8 % crude protein) and fish meal (58 % crude protein) were provided by Nicoluzzi Rações Ltda; [3]Contained 62 % crude protein and was produced by IMCOPA S.A.; [4]Produced by Bunge Alimentos S.A.; [5]Composition per kg product: dicalcium phosphate 565 g, potassium chloride 60 g, sodium chloride 65 g, and magnesium sulphate 310 g; [6]Choline bitartrate (1.0 g kg^{-1}) and vitamin-micromineral premix (produced by Cargill), composition per kg: folic acid 420 mg, pantothenic acid 8333 mg, BHT 25,000 mg, biotin 134 mg, cobalt sulphate 27 mg, copper sulphate 1,833 mg, iron sulphate 8,000 mg, calcium iodate 92 mg, manganese sulphate 3,500 mg, niacin 8.333 mg, selenite 100 mg, vitamin (vit.) A 1,666,670 UI, vit. B_1 2083 mg, vit. B_{12} 5,000 µg, vit. B_2 4,166 mg, vit. B_6 3166 mg, ascorbic acid equivalent 66,670 mg, vit. D_3 666,670 UI, vit. E 16,666 UI, vit. K_3 833 mg, zinc sulphate 23,330 mg, inositol 50,000 mg, and calcium propionate 250,000 mg; [7]yttrium (III) oxide – 99.9 % trace metal basis. Sigma-Aldrich.

moisture of the ingredient mixture was adjusted to 25 % using water. Diet extrusion was performed in a single-screw extruder (model MX-40; Inbramaq). The extrusion parameters were as follows: temperature 100 °C; thread speed 220 rpm; flow rate 20 % of rated capacity; width to diameter ratio 2.3:1; thread diameter 92.5 mm; and cylinder length 210 mm. After extrusion, pellets (4 mm) were dried in a forced-air circulation oven at 55 °C and then packaged and stored in air-tight containers at a constant temperature of 23 °C. The proximate and AA compositions of the two reference and the four experimental diets are shown in Tables 2 and 3, respectively.

Fish and experimental conditions

The digestibility trial was performed in Florianópolis, Santa Catarina State, Brazil (27°43′45″ S, 48°30′31″ W, altitude 3 m), following protocol 9377080618 approved by the Ethics Committee on Animal Use of the Federal University of Santa Catarina (CEUA, UFSC). Nile tilapia juveniles of the GIFT strain, sexually inverted to male, were obtained from a commercial fish farm (Piscicultura Pomerode). Before the digestibility trial, fish were acclimated to laboratory conditions for two weeks in three 1,000 L tanks connected to a recirculation system and equipped with biological and mechanical filtration, an air supply, and a heat exchanger. The temperature was set to 28 °C, and the photoperiod was adjusted to 12 h.

Table 2 – Analyzed nutritional composition of the reference diets and test ingredients.

Composition (g kg^{-1} dry matter)	Reference diet		Test ingredients	
	Semi-purified	Practical	Soybean meal[1]	Poultry by-product meal[2]
Dry matter	877.40	904.10	882.70	963.40
Crude protein	385.23	352.28	544.60	671.10
Ether extract	68.30	65.40	22.60	171.20
Neutral detergent fibre	73.20	66.30	166.02	-
Ash	22.80	74.20	73.60	145.30
Gross energy (kcal kg^{-1})	4858	4451	4863	5396
Energy:Protein	12.61	12.63	-	-
Essential amino acids				
Arginine	18.50	23.30	37.00	44.50
Histidine	8.80	8.23	13.10	12.60
Isoleucine	16.30	14.50	23.20	24.60
Leucine	29.60	27.10	38.60	44.60
Lysine	26.51	19.10	30.50	36.90
Methionine	9.10	7.40	6.80	12.70
Phenylalanine	16.81	16.60	25.50	24.80
Threonine	14.10	13.14	19.50	25.00
Tryptophan	3.72	3.82	6.80	6.30
Valine	20.77	16.00	24.10	30.30
Non-essential amino acids				
Alanine	17.71	18.95	21.90	41.70
Aspartic acid	26.54	34.96	57.90	53.50
Cysteine	1.34	4.11	7.40	7.10
Glycine	28.62	21.44	21.40	62.40
Glutamic acid	72.62	57.20	90.90	84.30
Proline	43.74	21.36	25.40	41.20
Serine	19.64	16.50	25.30	28.20

[1]Nicoluzzi Rações, Ltda; [2]Kabsa S.A.

Table 3 – Analyzed nutritional composition of the test diets.

Composition (g kg^{-1} dry matter)	Soybean meal		Poultry by-product meal	
	Semi-purified	Practical	Semi-purified	Practical
Dry matter	905.80	908.80	888.60	914.60
Crude protein	422.72	402.62	485.03	457.36
Ether extract	58.50	59.50	66.80	92.20
Ash	40.10	75.40	65.90	102.00
Neutral detergent fibre	116.10	118.40	51.30	57.60
Gross energy (kcal g^{-1})	4864	4587	5019	4734
Energy:Protein	11.50	11.39	10.34	10.35
Essential amino acids				
Arginine	23.60	27.44	27.41	29.62
Histidine	10.00	9.70	10.62	9.90
Isoleucine	18.33	17.30	19.63	17.80
Leucine	32.23	30.72	35.40	32.54
Lysine	27.13	22.54	31.54	25.31
Methionine	8.00	7.71	10.65	7.73
Phenylalanine	19.70	19.72	20.03	19.00
Threonine	15.30	15.10	18.10	16.53
Tryptophan	4.70	4.72	4.80	4.65
Valine	21.90	18.60	24.40	20.63
Non-essential amino acids				
Alanine	18.73	19.90	26.30	26.40
Aspartic acid	35.50	41.90	36.30	40.80
Cysteine	2.90	4.90	2.52	4.52
Glycine	26.00	21.40	39.90	34.10
Glutamic acid	76.50	67.20	79.40	65.60
Proline	38.14	22.80	44.20	27.70
Serine	20.80	19.30	22.10	18.80

Following this period, fish with an average initial weight of 65.05 g ± 12.37 and a total length of 14.75 cm ± 0.86 (mean ± standard deviation) were transferred to 24 experimental units (115 L circular tanks), with biomass of approximately 1,500 g per unit (23 fish per unit). Tanks were connected to a closed freshwater recirculation system with aeration, mechanical and biological filtration, and temperature and photoperiod were adjusted to 28 °C and 12 h of light, respectively. The experiment was conducted in a completely randomized design, using four replications for each experimental diet. Fish were fed twice a day (10h00 and 16h00) until apparent satiation and fecal collection started 7 day after changing the experimental diets to allow the excretion of all previously ingested feed. Feces were collected within each experimental unit by siphoning for 30 day. One hour after the last feeding, the tanks were cleaned, and the total water volume was renewed to avoid contamination of newly voided feces with uneaten feed and old feces. After cleaning, the water flow was interrupted for one hour, and newly voided feces were collected by siphoning. The collected feces were lyophilized, homogenized, and stored at –20 °C until analysis.

The water quality indicators were measured weekly, except for temperature, dissolved oxygen, and the pH, which were monitored daily. The average values (± standard deviation) were as follows: temperature 27.96 ± 0.36 °C; dissolved oxygen 5.69 ± 0.41 mg L^{-1}; pH 7.42 ± 0.24; salinity 1.97 ± 0.20 g L^{-1}; alkalinity 59.82 ± 8.30 $CaCO_3$ mg L^{-1}; total ammonia 0.55 ± 0.10 mg L^{-1}; and 0.01 mg L^{-1} nitrite. All variables measured remained within the comfort range of Nile tilapia (Webster and Lim, 2006). The water inflow rate in each experimental unit was 25 mL s^{-1}.

Chemical analysis

The proximate analysis of the diet ingredients, diets, and feces followed procedures standardized by the "Association of Official Analytical Chemists" (AOAC, 1999): moisture (dried at 105 °C to a constant weight, method 950.01), total lipid (Soxhlet, method 920.39C), and ash (incineration at 550 °C, method 942.05). According to the manufacturer's instructions, gross energy was determined using a calorimeter (PARR, model ASSY 6200). Crude protein, the amino acid content, crude fiber, and neutral detergent fiber of the diet ingredients (corn, fish meal, soy protein concentrate, poultry by-product, and soybean meal) were analyzed using near-infrared spectroscopy (NIRs) at the Animal Nutrition Laboratory (Evonik).

Crude protein and amino acid from feed and feces were analyzed by wet chemistry at the Evonik Laboratory using ion-exchange chromatography with

post-column derivatisation with ninhydrin. Amino acids (AAs) were oxidized with performic acid and neutralized with sodium metabisulfite25 (Commission Directive 1998). AAs were liberated from the protein by hydrolysis with 6N HCl for 24 h at 110 °C and quantified using the internal standard method by measuring the absorption of reaction products with ninhydrin at 570 nm. The inert marker yttrium was measured using inductively coupled plasma mass spectrometry (ICP-MS) at the Atomic Spectrometry Laboratory (UFSC). The samples were introduced using a pneumatic nebulizer.

Calculation of apparent digestibility coefficient

Protein, AAs, and dry matter apparent digestibility coefficients (ADCs) were estimated using the following equations:

For the diets (NRC, 2011):

$$ADC_{nutr}\% = 100 - \left[100 \times \left(\frac{\%Marker_{Diet}}{\%Marker_{Feces}} \times \frac{Nutrient_{Feces}}{Nutriente_{Diet}}\right)\right]$$

For the test ingredients (Bureau et al., 1999):

$$ADC_{ing}\% = ADC_{td} + \left[\left(ADC_{td} - ADC_{ref}\right) \times \left[\frac{0.7 \times Nutrient_{ref}}{0.3 \times Nutriente_{ing}}\right]\right]$$

where nutr = nutrient, ing = ingredient, td = test diet, and ref = reference diet.

Statistical analysis

All data are reported as mean ± standard deviation. To test differences between the two types of reference diets, ADC data were first tested for normality and homoscedasticity and then subjected to the Student's *t*-test. The same procedures were applied to test the differences between the ADCs of the tested ingredients (SBM and PBM) within each different reference diet (SP and P). Statistical analyses were performed using Statistica 10.0 software, adopting a 5 % confidence level.

Results

The ADCs of dry matter, protein, energy, and AAs in the reference diets used in this study are presented in Table 4. The ADCs of dry matter and crude protein of the P reference diet were higher than those of the SP reference diet. The ADCs of most AAs exceeded 90 % and were similar between the reference diets. However, for the EAAs arginine and histidine and the non-essential amino acids (NEAA) aspartic acid and cysteine, the type of reference diet affected the ADC values, which were higher for the P reference diet. In turn, the ADC of leucine was higher in the SP reference diet. In both reference diets, the AAs arginine, lysine, and glutamic acid presented the highest ADC values (92.76 – 96.11 %), whereas isoleucine presented the lowest values (86.09 – 86.66 %).

Table 4 – Apparent digestibility coefficients of dry matter, protein, energy, and amino acids of the two reference diets for Nile tilapia.

Nutrient	Reference diet		*p*-value
	Semi-purified	Practical	
Dry matter	75.27 ± 2.64[b]	80.14 ± 0.55[a]	0.044
Crude protein	88.90 ± 0.25[b]	90.40 ± 0.43[a]	0.023
Energy	80.31 ± 0.38	82.08 ± 0.57	0.060
Essential amino acids			
Arginine	92.76 ± 0.97[b]	96.11 ± 0.18[a]	0.008
Histidine	90.05 ± 0.87[b]	92.89 ± 0.21[a]	0.010
Isoleucine	86.66 ± 1.21	86.09 ± 1.03	0.609
Leucine	90.32 ± 0.88[a]	87.84 ± 0.58[b]	0.029
Lysine	94.19 ± 0.79	94.79 ± 0.29	0.286
Methionine	92.48 ± 0.61	91.56 ± 0.34	0.111
Phenylalanine	89.25 ± 0.85	90.68 ± 0.26	0.061
Threonine	88.62 ± 1.08	87.84 ± 0.57	0.353
Valine	90.46 ± 0.81	89.87 ± 0.36	0.333
Non-essential amino acids			
Alanine	89.07 ± 1.04	90.34 ± 0.39	0.132
Aspartic acid	90.72 ± 1.05[b]	93.71 ± 0.32[a]	0.016
Cysteine	74.88 ± 2.25[b]	89.56 ± 0.33[a]	0.001
Glycine	89.52 ± 1.00	91.23 ± 0.59	0.088
Glutamic acid	93.15 ± 0.70	94.44 ± 0.19	0.050
Proline	91.88 ± 0.72	91.32 ± 0.36	0.318
Serine	92.63 ± 0.79	92.67 ± 0.21	0.936
Mean ADC for all AAs	89.81 ± 4.28	91.27 ± 2.63	

[a, b]Values followed by different superscripts within the same row are different.

The ADCs of selected nutrients in SBM tested using the two types of reference diets are presented in Table 5. The type of ingredients used in the reference diets did not affect the ADCs of dry matter, while ADC of protein was higher in the SBM tested using the SP reference diet than the P reference diet. Similarly, the ADCs for all AAs were higher in SBM when tested using the SP versus the P-type reference diet, except for cysteine, but with no difference. The EAAs arginine, histidine, and lysine, and NEAAs aspartic acid and glutamic acid exhibited the highest ADC values (95.21 – 103.41 %). In contrast, the EAA isoleucine exhibited the lowest ADC values (89.11 and 94.21 %, respectively, for the P and SP diets).

The ADCs of the selected nutrients of PBM tested using two reference diets are presented in Table 5. The ADCs of dry matter, protein, and most AAs were not affected by the type of reference diet. However, the ADCs of histidine, glycine, and proline were higher in the PBM-SP diet, whereas only the ADC of cysteine was substantially higher in the PBM-P diet than in the PBM-SP diet. For the latter test ingredient, the EAAs arginine, histidine, and lysine, and the NEAAs alanine, glycine, and glutamic acid exhibited the highest values of ADC (93.28 – 98.84 %), regardless of the type of reference diet.

Table 5 – Apparent digestibility coefficients of dry matter, protein, energy, and amino acids in soybean meal and poultry by-product meal for Nile tilapia.

Nutrient	Soybean meal		*p*-value	Poultry by-product meal		*p*-value
	Semi-purified	Practical		Semi-purified	Practical	
Dry matter	80.13 ± 1.44	78.40 ± 1.82	0.236	87.38 ± 1.11	86.26 ± 1.64	0.302
Crude protein	99.64 ± 1.12[a]	93.94 ± 1.51[b]	0.003	95.75 ± 1.70	94.63 ± 2.20	0.450
Energy	80.04 ± 0.42	80.75 ± 0.81	0.391	89.56 ± 0.17	90.27 ± 1.54	0.919
Essential amino acids						
Arginine	100.99 ± 1.16[a]	97.19 ± 0.53[b]	0.002	94.18 ± 1.10	93.70 ± 1.35	0.598
Histidine	101.92 ± 0.85[a]	95.21 ± 1.12[b]	0.000	97.07 ± 1.90[A]	93.29 ± 2.31[B]	0.045
Isoleucine	94.21 ± 1.64[a]	89.11 ± 2.24[b]	0.021	92.81 ± 2.62	94.70 ± 3.05	0.383
Leucine	97.11 ± 1.54[a]	92.00 ± 1.32[b]	0.005	92.93 ± 2.20	94.85 ± 2.90	0.333
Lysine	100.83 ± 1.04[a]	96.04 ± 0.72[b]	0.001	94.24 ± 1.41	93.28 ± 1.46	0.378
Methionine	103.35 ± 1.82[a]	93.62 ± 1.61[b]	0.001	94.16 ± 2.00	92.35 ± 1.90	0.236
Phenylalanine	99.44 ± 0.88[a]	93.49 ± 1.52[b]	0.002	94.71 ± 2.11	92.96 ± 2.72	0.348
Threonine	94.77 ± 0.97[a]	91.12 ± 2.13[b]	0.042	91.65 ± 2.00	93.26 ± 2.58	0.363
Valine	100.43 ± 1.12[a]	92.58 ± 1.84[b]	0.001	93.23 ± 2.03	92.72 ± 2.39	0.759
Non-essential amino acids						
Alanine	99.97 ± 2.41[a]	92.52 ± 2.25[b]	0.008	94.55 ± 1.64	94.31 ± 2.03	0.864
Aspartic acid	100.51 ± 0.76[a]	96.57 ± 1.02[b]	0.003	92.41 ± 1.54	91.83 ± 2.09	0.675
Cysteine	94.44 ± 0.66	93.02 ± 1.88	0.275	84.09 ± 2.33[B]	90.78 ± 2.88[A]	0.011
Glycine	104.71 ± 5.62[a]	90.32 ± 2.94[b]	0.007	98.84 ± 1.37[A]	95.35 ± 1.56[B]	0.015
Glutamic acid	103.41 ± 0.72[a]	97.51 ± 0.93[b]	0.000	96.40 ± 1.85	93.39 ± 1.85	0.061
Proline	107.52 ± 2.60[a]	93.87 ± 1.81[b]	0.000	103.90 ± 1.78[A]	96.11 ± 1.72[B]	0.001
Serine	100.70 ± 0.97[a]	94.65 ± 1.40[b]	0.001	92.94 ± 2.14	93.49 ± 2.17	0.731
Mean ADC for all AAs	100.19 ± 3.87	93.66 ± 2.74		94.10 ± 4.23	93.43 ± 2.36	

[a, b]For soybean meal: within the same row, values followed by different letters are different; [A, B]For poultry by-products, within the same row, values followed by different letters are different.

Discussion

The correct assessment of nutrient use for a species of great economic importance, such as Nile tilapia, is essential to formulate efficient diets. Several strategies can be applied to assess the nutritional quality of ingredients; however, the choice of these strategies can affect data interpretation (Glencross, 2020). The composition of the reference diet varies greatly in digestibility studies for Nile tilapia from those using only practical (Cardoso et al., 2021; Guimarães et al., 2008; Hernandéz et al., 2010; Köprücü and Özdemir, 2005; Magalhães et al., 2018; Schneider et al., 2004; Vidal et al., 2015, 2017) or SP type ingredients (Borghesi et al., 2008; Furuya et al., 2001; Rodrigues et al., 2012) to those using a mixture of both (Davies et al., 2011; Xavier et al., 2014). Nevertheless, data on the ADCs of nutrients within reference diets are challenging to interpret or discuss. Our findings showed that the type of ingredients in the reference diet could affect the ADCs of dry matter, protein, and selected AAs.

For both types of reference diets, digestibility of protein and AAs was similar, with minor variations in ADCs, except for cysteine. Four AAs (arginine, histidine, aspartic acid, and cysteine) were more digestible in the P reference diet. Leucine was the only AA with a high ADC in the SP reference diet. Therefore, these differences suggested that the digestibility of a particular AA depends on the protein type that composes the P or SP reference diet and the capacity of Nile tilapia to digest each particular AA, considering their chemical characteristics. Arginine and histidine are positively charged (basic) AAs, whereas aspartic acid is a negatively charged (acidic) AA under physiological conditions, with both types mostly exposed to the protein surface. These charged R-groups are more hydrophilic, facilitating enzyme-catalyzed reactions by functioning as proton donors and acceptors (Nelson and Cox, 2017). Considering our results and that a reference diet with P ingredients is more palatable than the SP type (NRC, 2011), we speculated that the highest feed intake, promoted by the greatest diet palatability, increases the activity of digestive enzymes (Moraes and Almeida, 2020). Although our findings exhibited similarities between the protein ADC and the average AA coefficients, individual AA ADCs are variable and can be higher or lower than the coefficient value for protein digestibility (NRC, 2011; Storebakken et al., 2000), as also observed in our study.

SBM exhibited very high digestibility for protein (93.94 % and 99.64 %), with most AA ADCs exceeding 93 %, regardless of the type of reference diet used. These findings were similar to those previously reported for Nile tilapia: 92.72 % (Furuya et al., 2001), 91.56 % (Pezzato et al., 2002), and 92.74 % (Guimarães et al., 2008), but higher than that reported by Ribeiro et al. (2011) (86.01 %) that used the dissection method for fecal collection. A comparison of the ADCs of SBM using different reference diets showed that SBM-SP resulted in a higher ADC of dry matter (80.13 %) and protein (99.64 %) than SBM-P (78.40 % and 93.94 %, respectively). The AA digestibility values followed the same pattern, with mean ADCs of 100.19 % and 93.66 % for SBM-SP and SBM-P diets, respectively. A similar result for the average AA ADC (92.30 %) was reported by Guimarães et al. (2008), while a lower value (87.10 %) was reported by Köprücü and Özdemir (2005), who used the same combination of SBM and a P-type reference diet.

The variation in our study regarding protein and AA digestibility of SBM could be explained by the differences in diet palatability and subsequent feed intake, anti-nutritional factors, and endogenous nitrogen (N) losses. The lower palatability of the SP ingredients compared to the P ingredients could partially explain such discrepancies. Moreover, SBM contains anti-nutritional factors that negatively affect its palatability to fish compared to animal sources, such as fish meal or PBM (Gatlin et al., 2007; NRC, 2011). The SP reference diet and SBM combination resulted in reduced feed intake, as evident in our trial. Fish were fed twice a day to apparent satiation, and during daily feeding (although feed intake was not recorded), fish fed with the P diet combinations exhibited greater voracity.

Another important factor in nutrient use is the speed at which the feed passes through the digestive tract because transit time has been reported to affect nutrient utilization efficiency (Elesho et al., 2021; Henken et al., 1985; NRC, 2011; Riche et al., 2004). When fish consumes less feed, as evident from the SBM-SP diet, the transit time is lowered, and the feed is subjected to more prolonged digestion exposure, resulting in increased absorption (Moraes and Almeida, 2020). In addition to palatability, the absence of anti-nutritional factors in SP ingredients made them more digestible to fish, thereby reducing the need for high feed intake. Phytate, an anti-nutritional present in plant feedstuffs such as soybean meal, cannot be digested by fish (Kumar et al., 2012; Oliva-Teles et al., 1998; Rodehutscord and Pfeffer, 1995). Besides, the deleterious effect of phytate on phosphorous availability also inhibits proteases, such as trypsin and pepsin (Liu et al., 2009), decreasing protein and AA digestibility (Lima et al., 2021; Spinelli et al., 1983). The presence of phytate in plant ingredients composing the P reference diet could explain the lower ADC of protein and AAs in the SMB-P diet compared to the SBPM-SP diet.

A possible reason for the higher ADC values in the SBM obtained when feeding the SP diet is the endogenous loss that occurs during digestion. The primary sources of N endogenous AA losses in animals are proteins that are endogenously synthesized and secreted in the digestive tract but are not digested and re-absorbed (Nyachoti et al., 1997). Endogenous losses can be induced by ingestion of a diet with a particular composition, such as protein level and fibre type (Cowieson and Ravindran, 2007; Stein et al., 1999). SP and P ingredients have distinct nutritional compositions and properties that directly affect the final characteristics of experimental diets. Although the nutrient contents of the reference diets were similar, the protein type (casein and gelatin *versus* fish meal and soy protein concentrate) and the fibre type (cellulose *versus* corn) have distinct characteristics. They can affect endogenous loss by changing viscosity and ingesta transit speed, which can affect mucin secretion and epithelial cell turnover (Parsons et al., 1983; Sauer et al., 1991). The highest ADC of AAs in the SBM-SP diet suggests that the ingredients used in the SP reference diet reduced endogenous AA losses. This may occur during the digestive process because of the absence of anti-nutritional factors in the SP ingredients and the interaction between dietary nutrients, such as fiber and protein.

Notably, regardless of the type of reference diet tested, the AAs arginine, histidine, lysine, aspartic acid, and glutamic acid presented the highest ADC values (> 95 %) in SBM. This is probably because they are charged AAs, highly hydrophilic, and more susceptible to enzyme reactions. Possible explanations for coefficients above 100 % include analytical errors for nutrients and markers, sampling, improper diet mixing, or interactions between diet ingredients (Glencross et al., 2007). We suggest that the SP ingredients, in addition to not having anti-nutritional factors and reducing endogenous AA losses, may interact with the nutrients contained in the SBM leading to overestimations of the final ADC values of some AAs. The digestibility of all EAAs was high in both types of reference diet, confirming the applicability of this ingredient as a vegetable source of digestible EAAs in aquafeeds, as previously reported by Elesho et al. (2021) and Vidal et al. (2017).

The nutrient digestibility values for PBM revealed less influence from the reference diet composition. Data showed high digestibility of dry matter (99.73 % and 99.74 %), protein (94.63 and 95.75 %), and most of the AAs (> 93 %). The mean AA ADCs were 94.10 % and 93.43 % for PBM-SP and PBM-P, respectively, similar to the mean 91.20 % reported by Guimarães et al. (2008). PBM is considered one of the most promising alternatives to replace fish meal because of its high protein content and quality, AA profile, essential fatty acids, vitamins, minerals, and good palatability (Cruz-Suárez et al., 2007; Gunben et al., 2014). The nutrient ADC of PBM confirmed the applicability of this ingredient in aquafeeds for Nile tilapia and other omnivorous species, such as African

catfish (*Clarias gariepinus*) and Pacific white shrimp (*Litopenaeus vannamei*), as reported by Elesho et al. (2021) and Cruz-Suárez et al. (2007), respectively. We did not observe a reduction in voracity in Nile tilapia when PBM was tested in an SP reference diet. The good palatability of animal protein sources could have minimized the effects of a non-attractive SP reference diet as registered with the plant ingredient SBM. Thus, the potential effects of an SP reference diet to increase the ADC values, verified when testing SBM, were observed in minor proportions when testing PBM because only three of the 16 AAs evaluated presented higher ADCs.

Conclusions

As a typical omnivorous species, Nile tilapia exhibits a high capacity to digest different protein-rich ingredients (SBM and PBM), with most ADCs exceeding 90 %. Our findings demonstrate that the type of ingredients used in the reference diet (SP or P) affects the plant protein source SBM more significantly compared to the animal protein source PBM. Thus, using practical ingredients in the reference diet has more relevance and can be applied in digestibility studies for Nile tilapia considering, diet palatability, amount of feces collected, costs, and availability.

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Authors' Contributions

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References

Association of Official Agricultural Chemists – International [AOAC]. 1999. Official Methods of Analysis of the Association of Official Analytical Chemists. 17ed. AOAC. Washington, DC, USA.

Borghesi, R.; Portz, L.; Oetterer, M.; Cyrino, J.E.P. 2008. Apparent digestibility coefficient of protein and amino acids of acid, biological and enzymatic silage for Nile tilapia (*Oreochromis niloticus*). Aquaculture Nutrition 14: 242-248. https://doi.org/10.1111/j.1365-2095.2007.00523.x

Bureau, D.P.; Harris, A.M.; Cho, C.Y. 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 180: 345-358. https://doi.org/10.1016/S0044-8486(99)00210-0

Cardoso, M.S.; Godoy, A.C.; Oxford, J.H.; Rodrigues, R.; Cardoso, M.S.; Bittencourt, F.; Signor, A.; Boscolo, W.R.; Feiden, A. 2021. Apparent digestibility of protein hydrolysates from chicken and swine slaughter residues for Nile tilapia. Aquaculture 530: 735720. https://doi.org/10.1016/j.aquaculture.2020.735720

Cowieson, A.J.; Ravindran, V. 2007. Effect of phytic acid and microbial phytase on the flow and amino acid composition of endogenous protein at the terminal ileum of growing broiler chickens. British Journal of Nutrition 98: 745-752. https://doi.org/10.1017/s0007114507750894

Cruz-Suárez, L.E.; Nieto-López, M.; Guajardo-Barbosa, C.; Tapia-Salazar, M.; Scholz, U.; Ricque-Marie, D. 2007. Replacement of fish meal with poultry by-product meal in practical diets for *Litopenaeus vannamei*, and digestibility of the tested ingredients and diets. Aquaculture 272: 466-476. https://doi.org/10.1016/j.aquaculture.2007.04.084

Davies, S.J.; Abdel-Warith, A.A.; Gouveia, A. 2011. Digestibility characteristics of selected feed ingredients for developing bespoke diets for Nile tilapia culture in Europe and North America. *Journal of the World Aquaculture Society* 42: 388-398. https://doi.org/10.1111/j.1749-7345.2011.00478.x

Elesho, F.E.; Kröckel, S.; Sutter, D.A.H.; Nuraini, R; Chen, I.J.; Verreth, J.A.J.; Schrama, J.W. 2021. Effect of feeding level on the digestibility of alternative protein-rich ingredients for African catfish (*Clarias gariepinus*). Aquaculture 544: 737108. https://doi.org/10.1016/j.aquaculture.2021.737108

Food and Agriculture Organization [FAO]. 2022a. The State of World Fisheries and Aquaculture 2022: Towards Blue Transformation. FAO, Rome, Italy. https://doi.org/10.4060/cc0461en

Food and Agriculture Organization [FAO]. 2022b. FishStatJ: Universal Software for Fishery Statistical Time Series: Aquaculture Production 1950-2020. FAO, Rome, Italy. Available at: https://www.fao.org/fishery/en/statistics [Accessed Sept 20, 2022]

Fracalossi, D.M.; Cyrino, J.E.P. 2013. Nutriaqua: Nutrition and Feeding of Species of Interest to Brazilian Aquaculture = Nutriaqua: Nutrição e Alimentação de Espécies de Interesse para a Aquicultura Brasileira. 2ed. Sociedade Brasileira de Aquicultura e Biologia Aquática. Florianópolis, SC, Brazil (in Portuguese).

Furuya, W.M.; Pezzato, L.E.; Pezzato, A.C.; Barros, M.M.; Miranda, E.C. 2001. Digestibility coefficients and digestible amino acids values of some ingredients for Nile tilapia (*Oreochromis niloticus*). Revista Brasileira de Zootecnia 30: 1143-1149 (in Portuguese, with abstract in English). https://doi.org/10.1590/S1516-35982001000500002

Gatlin, D.M.; Barrows, F.T.; Brown, P.; Dabrowski, K.; Gaylord, T.G.; Hardy, R.W.; Herman, E.; Hu, G.; Krogdahl, Å.; Nelson, R.; Overturf, K.; Rust, M.; Sealey, W.; Skonberg, D.; Souza, E.J.; Stone, D.; Wilson, R.; Wurtele, E. 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquaculture Research 38: 551-579. https://doi.org/10.1111/j.1365-2109.2007.01704.x

Glencross, B.D.; Booth, M.; Allan, G.L. 2007. A feed is only as good as its ingredients - a review of ingredient evaluation strategies for aquaculture feeds. Aquaculture Nutrition 13: 17-34. https://doi.org/10.1111/j.1365-2095.2007.00450.x

Glencross, B.D. 2020. A feed is still only as good as its ingredients: an update on the nutritional research strategies for the optimal evaluation of ingredients for aquaculture feeds. Aquaculture Nutrition 26: 1871-1883. https://doi.org/10.1111/anu.13138

Guimarães, I.G.; Pezzato, L.E.; Barros, M.M. 2008. Amino acid availability and protein digestibility of several protein sources for Nile tilapia, *Oreochromis niloticus*. Aquaculture Nutrition 14: 396-404. https://doi.org/10.1111/j.1365-2095.2007.00540.x

Gunben, E.M.; Senoo, S.; Yong, A.S.K.; Shapawi, R. 2014. High potential of poultry by-product meal as a main protein source in the formulated feeds for a commonly cultured grouper in Malaysia (*Epinephelus fuscoguttatus*). Sains Malaysiana 43: 399-405.

Hardy, R.W.; Barrows, F.T. 2002. Fish Nutrition. Academic Press, Cambridge, MA, USA.

Hardy, R.W. 2010. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquaculture Research 41: 770-776. https://doi.org/10.1111/j.1365-2109.2009.02349.x

Henken, A.M.; Kleingeld, D.W.; Tijssen, P.A.T. 1985. The effect of feeding level on apparent digestibility of dietary dry matter, crude protein and gross energy in the African catfish *Clarias gariepinus* (Burchell, 1822). Aquaculture 5: 1-11. https://doi.org/10.1016/0044-8486(85)90235-2

Hernandéz, C.; Olvera-Novoa, M.A.; Hardy, R.W.; Hermosillo, A.; Reyes, C.; González, B. 2010. Complete replacement of fish meal by porcine and poultry by-product meals in practical diets for fingerling Nile tilapia *Oreochromis niloticus*: digestibility and growth performance. Aquaculture Nutrition 16: 44-53. https://doi.org/10.1111/j.1365-2095.2008.00639.x

Köprücü, K.; Özdemir, Y. 2005. Apparent digestibility of selected feed ingredients for Nile tilapia (*Oreochromis niloticus*). Aquaculture 250: 308-316. https://doi.org/10.1016/j.aquaculture.2004.12.003

Kumar, V.; Sinha, A.K.; Makkar, H.P.S.; De Boeck, G.; Becker, K. 2012. Phytate and phytase in fish nutrition. Journal of Animal Physiology and Animal Nutrition 96: 335-364. https://doi.org/10.1111/j.1439-0396.2011.01169.x

Lima, G.S.; Lima, M.R.; Gomes, G.A.; Cavalcante, D.T.; Guerra, R.R.; Silva, J.H.V.; Cardoso, A.S.; Kaneko, I.N.; Costa, F.G.P. 2021. Superdosing of bacterial phytase (EC 3.1.3.26) in broiler diets with reduced levels of digestible amino acids. Livestock Science 253: 104714. https://doi.org/10.1016/j.livsci.2021.104714

Liu, N.; Ru, Y.J.; Li, F.D.; Wang, J.P.; Lei, X.Q. 2009. Effect of dietary phytate and phytase on proteolytic digestion and growth regulation of broilers. Archives of Animal Nutrition 63: 292-303. https://doi.org/10.1080/17450390903020422

Lovell, R.T. 1998. Nutrition and Feeding of Fish. 2ed. Kluwer Academic Press. Boston, MA, USA.

Lupatsch, I.; Kissil, G.W.M.; Sklan, D.; Pfeffer, E. 1997. Apparent digestibility coefficients of feed ingredients and their predictability in compound diets for gilthead seabream, *Sparus aurata* L. Aquaculture Nutrition 3: 81-89. https://doi.org/10.1046/j.1365-2095.1997.00076.x

Maas, R.M.; Verdegem, M.C.J.; Schrama, J.W. 2019. Effect of non-starch polysaccharide composition and enzyme supplementation on growth performance and nutrient digestibility in Nile tilapia (*Oreochromis niloticus*). Aquaculture Nutrition 25: 622-632. https://doi.org/10.1111/anu.12884

Magalhães, S.C.Q.; Cabrita, A.R.J.; Valentão, P.; Andrade, P.B.; Rema, P.; Maia, M.R.G.; Valente, L.M.P.; Fonseca, A.J.M. 2018. Apparent digestibility coefficients of European grain legumes in rainbow trout (*Oncorhynchus mykiss*) and Nile tilapia (*Oreochromis niloticus*). Aquaculture Nutrition 24: 332-340. https://doi.org/10.1111/anu.12564

Moraes, G.; Almeida, L.C. 2020. Nutrition and functional aspects of digestion in fish. p. 251-271. In: Baldisserotto, B.; Cyrino, J.E.P.; Urbinati, E.C. eds. Biology and physiology of freshwater neotropical fish. Academic Press, Cambridge, MA, USA. https://doi.org/10.1016/B978-0-12-815872-2.00011-7

National Research Council [NRC]. 2011. Nutrient Requirements of Fish and Shrimp. National Academic Press. Washington, DC, USA.

Nelson, D.L.; Cox, M.M. 2017. Lehninger Principles of Biochemistry. 7ed. W.H. Freeman, New York, NY, USA.

Nguyen, T.N.; Davis, D.A.; Saoud, I.P. 2009. Evaluation of alternative protein sources to replace fish meal in practical diets for juvenile tilapia, *Oreochromis* spp. Journal of the World Aquaculture Society 40: 113-121. https://doi.org/10.1111/j.1749-7345.2008.00230.x

Nyachoti, C.M.; Lange, C.F.M.; McBride, B.W.; Schulze, H. 1997. Significance of endogenous gut nitrogen losses in the nutrition of growing pigs: A review. Canadian Journal of Animal Science 77: 149-163. https://doi.org/10.4141/A96-044

Oliva-Teles, A.; Pereira, J.P.; Gouveia, A.; Gomes, E. 1998. Utilization of diets supplemented with microbial phytase by seabass (*Dicentrarchus labrax*) juveniles. Aquatic Living Resources 11: 255-259 https://doi.org/10.1016/S0990-7440(98)80008-9

Parsons, C.M.; Potter, L.M.; Brown Jr., R.D. 1983. Effects of dietary carbohydrate and of intestinal microflora on excretion of endogenous amino acids by poultry. Poultry Science 62: 483-489. https://doi.org/10.3382/ps.0620483

Pezzato, L.E.; Miranda, E.C.; Barros, M.M.; Pinto, L.G.Q.; Furuya W.M.; Pezzato, A.C. 2002. Apparent digestibility of feedstuffs by Nile tilapia (*Oreochromis niloticus*). Revista Brasileira de Zootecnia 31: 1595-1604 (in Portuguese, with abstract in English). https://doi.org/10.1590/S1516-35982002000700001

Pezzato, L.E.; Barros, M.M.; Boscolo, W.R.; Cyrino, J.E.P.; Furuya, V.R.B.; Feiden, A. 2010. Brazilian Tables for Tilapia's Nutrition = Tabelas Brasileiras para a Nutrição de Tilápias. GFM, Toledo, PR, Brazil (in Portuguese).

Ribeiro, F.B.; Lanna, E.A.T.; Bomfim, M.A.D.; Donzele, J.L.; Quadros, M.; Cunha, P.S.L. 2011. True and apparent digestibility of protein and amino acids of feed in Nile tilapia. Revista Brasileira de Zootecnia 40: 939-946. https://doi.org/10.1590/S1516-35982011000500001

Riche, M.; Haley, D.I.; Oetker, M.; Garbrecth, S.; Garling, D.L. 2004. Effect of feeding frequency on gastric evacuation and the return of appetite in tilapia *Oreochromis niloticus* (L.). Aquaculture 234: 657-673. https://doi.org/10.1016/j.aquaculture.2003.12.012

Rodehutscord, M.; Pfeffer, E. 1995. Effects of supplemental microbial phytase on phosphorus digestibility and utilization in rainbow trout (*Oncorchynchus mykiss*). Water Science and Technology 31: 143-147. https://doi.org/10.1016/0273-1223(95)00433-N

Rodrigues, A.P.O.; Gominho-Rosa, M.D.C.; Ferreira, C.E.; Francisco, A.; Fracalossi, D.M. 2012. Different utilization of plant sources by the omnivores jundiá catfish (*Rhamdia quelen*) and Nile tilapia (*Oreochromis niloticus*). Aquaculture Nutrition 18: 65-72. https://doi.org/10.1111/j.1365-2095.2011.00877.x

Sauer, W.C.; Mosenthin, R.; Ahrens, F.; Den Hartog, L.A. 1991. The effect of source of fiber on ileal and fecal amino acid digestibility and bacterial nitrogen excretion in growing pigs. Journal of Animal Science 69: 4070-4077. https://doi.org/10.2527/1991.69104070x

Schneider, O.; Amirkolaie, A.K.; Vera-Cartas, J.; Eding, E.H.; Schrama, J.W.; Verreth, J.A.J. 2004. Digestibility, faeces recovery, and related carbon, nitrogen and phosphorus balances of five feed ingredients evaluated as fishmeal alternatives in Nile tilapia, *Oreochromis niloticus* L. Aquaculture Research 35: 1370-1379. https://doi.org/10.1111/j.1365-2109.2004.01179.x

Spinelli, J.; Houle, C.R.; Wekell, J.C. 1983. The effect of phytates on the growth of rainbow trout (*Salmo gairdneri*) fed purified diets containing varying quantities of calcium and magnesium. Aquaculture 30: 71-83. https://doi.org/10.1016/0044-8486(83)90153-9

Stein, H.H.; Trottier, N.L.; Bellaver, C.; Easter, R.A. 1999. The effect of feeding level and physiological status on total flow and amino acid composition of endogenous protein at the distal ileum in swine. Journal of Animal Science 77: 1180-1187. https://doi.org/10.2527/1999.7751180x

Storebakken, T.; Shearer, K.D.; Baeverfjord, G.; Nielsen, B.G.; Åsgård, T.; Scott, T.; De Laportet, A. 2000. Digestibility of macronutrients, energy and amino acids, absorption of elements and absence of intestinal enteritis in Atlantic salmon, *Salmo salar*, fed diets with wheat gluten. Aquaculture 184: 115-132. https://doi.org/10.1016/S0044-8486(99)00316-6

Vidal, L.V.O.; Xavier, T.O.; Michelato, M.; Martins, E.N.; Prezzato, L.E.; Furuya, W.M. 2015. Apparent protein and energy digestibility and amino acid availability of corn and co-products in extruded diets for Nile tilapia, *Oreochromis niloticus*. Journal of the World Aquaculture Society 46: 183-190. https://doi.org/10.1111/jwas.12184

Vidal, L.V.O.; Xavier, T.O.; Moura, L.B.; Graciano, T.S.; Martins, E.N.; Furuya, W.M. 2017. Apparent digestibility of soybean coproducts in extruded diets for Nile Tilapia, *Oreochromis niloticus*. Aquaculture Nutrition 23: 228-235. https://doi.org/10.1111/anu.12383

Webster, C.D.; Lim, C. 2006. Tilapia: Biology, Culture, and Nutrition. CRC Press, Boca Raton, FL, USA.

Xavier, T.O.; Michelato, M.; Vidal, L.V.O.; Furuya, V.R.B.; Furuya, W.M. 2014. Apparent protein and energy digestibility and amino acid availability of commercial meat and bone meal for Nile tilapia, *Oreochromis niloticus*. Journal of the World Aquaculture Society 45: 439-446. https://doi.org/10.1111/jwas.12127